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Does Periconceptional Multivitamin Use Reduce the Risk of Neural Tube Defects Associated With Other Birth Defects? Data From Two Population-Based Case-Control Studies

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The role of periconceptional folic acid in the prevention of neural tube defects (NTDs) is well established. However, it is not clear whether a protective effect exists for the subset of nonsyndromic NTD with other "unrelated" major structural birth defects (NTD-multiples). This question is important to investigate because of shared pathogenetic mechanisms between NTD and other types of birth defects, and because of the epidemiologic differences that have been shown between NTD-multiples and NTD-singles. We analyzed data from two population-based case-control studies of NTDs, Atlanta 1968–1980, and California 1989–1991, to assess whether periconceptional multivitamin use reduces the risk of NTD-multiples. Maternal vitamin histories were assessed for 47 and 65 NTD-multiples cases and 3,029 and 539 control babies in Atlanta, and California, respectively. There was a substantial risk reduction associated with periconceptional multivitamin use (−3 to +3 months) for NTD-multiples (pooled odds ratio = 0.36, 95% C.I. 0.18–0.72) that persisted after adjustment for maternal race/ethnicity and education. Also, no specific types of NTDs or NTDs with specific defects explained the risk reduction with vitamin use. These data suggest that multivitamins reduce the risk of nonsyndromic NTD cases associated with other major birth defects. The implication of this finding for the role of vitamins in the prevention of non-NTD birth defects should be further explored.

KEY WORDS: abnormalities, folic acid, neural tube defects, prevention and control, vitamins

INTRODUCTION

The role of periconceptional folic acid supplementation in the prevention of neural tube defects (NTDs) has been well established [Eskes and Steegers-Theunissen, 1994]. As a result of the randomized clinical trials conducted both in recurrence and occurrence settings [MRC, 1991; Czeizel and Dudas; 1992], and the results of several observational studies [Mulinare et al., 1988; Milunsky et al., 1989; Bower and Stanley, 1989; Werler et al., 1993; Shaw et al., 1995], the U.S. Public Health Service now recommends that all women of childbearing age take 0.4 mg folic acid daily to reduce the risk of a NTD-affected pregnancy [CDC, 1992].

Nevertheless, since most NTD cases occur isolated, it is not clear whether a protective effect exists for the subset of nonsyndromic NTD cases that have accompanying "unrelated" major structural birth defects (NTD-multiples). While some NTD cases have associated anomalies that are components of an NTD embryonic sequence (e.g., clubfeet or hip dislocation), or part of a recognized syndrome (phenotypes of known cause such as the Mendelian disorder, Meckel syndrome, or chromosomal abnormalities such as trisomy 18) [Jones, 1988], it is well known that a proportion of NTD cases (ranges from 6% to 50%) have associated structural birth defects [Elwood et al., 1992]. A protective effect of folic acid among NTD-multiples is important to investigate because: (1) some studies have suggested that NTD-isolated and NTD-multiples have different epidemiologic characteristics (such as race, gender and secular trend variation) as well as recurrence risk estimates [e.g., Khoury et al., 1982a,b; Holmes et al., 1976;

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Martin et al., 1983]. However, these epidemiologic differences have not been observed in all studies [e.g., Shaw et al., 1994; Martinez-Frias et al., 1986]; and (2) NTDs may share similar pathogenetic mechanisms with other types of birth defects [Czeizel, 1981; Khoury et al., 1988; Opitz and Gilbert, 1982; Opitz, 1993]. NTDs seem to cluster with some other defects more than by chance. In addition, recent studies have suggested that there may be protective effects of periconceptional multivitamins for several non-NTD defects [Shaw et al., 1994a,b, Czeizel, 1993].

Because of the potential implications of these findings for prevention, we decided: (1) to assess whether multivitamin use can reduce the risk of nonsyndromic NTDs that are associated with other birth defects, and (2) to evaluate which types of associated birth defects share a risk reduction with NTDs. This latter inquiry may shed some light on which defects share pathogenetic similarities with NTDs. Because of the relatively low prevalence of NTD-multiples, we combined data from two large population-based case-control studies to address these questions.

METHODS

The present study is based on pooling data from two population-based case-control studies of neural tube defects conducted in California and Atlanta.

Study Populations

The California case-control study is based on cases of NTDs ascertained in the California Birth Defects Monitoring Program (CBDMP) with birth years between June 1, 1989 and May 31, 1991. The CBDMP is a population-based birth defects registry that uses multiple sources of case ascertainment. Women who had liveborn and stillborn infants with NTDs, as well as those who had NTD-affected pregnancies that were terminated after prenatal diagnosis, were enrolled in this study from a population base of over 700,000 births. An equal number of control infants were randomly selected (without one-to-one matching) from each area hospital in proportion to each hospital's contribution to the total birth population. Completed maternal interviews were available on 88% and 89% of eligible cases and controls, respectively. Most interviews were done within 6 months of delivery. Details of the study design are available elsewhere [Shaw et al., 1995].

The Atlanta data are derived from a larger case-control study of all major defects ascertained in the Metropolitan Atlanta Congenital Defects Program (MACDP) with birth years between 1968 and 1980, inclusive. The MACDP is a population-based birth defects registry that uses multiple sources of case ascertainment. Women who had liveborn and stillborn infants with NTDs were enrolled in this study from a population base of over 300,000 births. No elective pregnancy terminations were included in this study. Controls were a 1% random sample of live births selected from area hospitals. Controls were frequency-matched to babies with birth defects by race, hospital of birth and period of birth. Completed maternal interviews were available for 67% and 71% of eligible cases and controls, respectively. Maternal interviews were conducted in 1982 and 1983. Details of the study design are available elsewhere [Mulinare et al., 1988].

Outcome Classification

In both studies, NTDs included cases of anencephaly, spina bifida. Cases of encephalocele were included in the Atlanta study only. In addition, there were only a few cases of rare forms of NTDs including craniorachischisis and iniencephaly. Using all available anatomic information on cases, including hospital records, pathology and surgery reports, NTD cases were classified into 5 groups:

(1) Isolated. This group included cases with no major or unrelated anomalies. Infants with only minor defects or defects that could be considered a sequence to NTDs were included in this group. We used criteria for classification for minor and sequence defects that were published recently [see appendix, Khoury et al., 1994] by the International Clearinghouse for Birth Defects Monitoring Systems (ICBDMS). Examples of defects considered secondary to or part of NTD sequence were clubfoot, hip dislocation, adrenal and pituitary hypoplasia, small penis, and hydrocephalus. Isolated NTD cases were excluded from most analyses.

(2) Known syndromes. This category includes NTD cases that are associated with a known cause (single gene disorder such as Meckel syndrome or chromosomal abnormalities).

(3) Amniotic bands. A few cases had evidence of amniotic bands. These were separately classified since the genesis of NTD may be different from other types.

(4) Holoprosencephaly. There were a few NTD cases that also had evidence of holoprosencephaly. These were classified separately since the genesis of the NTD may be different from other types.

(5) Multiples. This group is the focus of the present study and consists of NTD cases that have associated major defects and that do not fit into any of the categories listed above. A final determination of multiples status was determined by two of us (M.J.K. and C.A.M.) for both study populations without knowledge of periconceptional vitamin exposure status. Associated defects were coded using a simple category coding scheme used by the ICBDMS [Khoury et al., 1994].

Assessment of Maternal Multivitamin Use

In California, questionnaires were administered primarily in person to mothers of cases and controls. Women were asked whether they used vitamin/mineral supplements in any of four time periods: 3 months prior to conception (−3 to 0); 3 months postconception (0 to +3); and the subsequent two trimesters of pregnancies. Details about type, frequency of use and brand were asked. There was also a dietary questionnaire designed to estimate daily folate intake. We also inquired about various other familial, medical, occupational, social and environmental exposures.

In Atlanta, questionnaires were administered over the telephone to mothers of cases and controls. Women were asked whether they used vitamins regularly (at least three times a week) at any time during the period

from 3 months prior to pregnancy to 3 months after pregnancy began (−3 to +3). Details about timing, type, frequency and brand were asked. No dietary questionnaire was given to estimate daily folate intake. We also inquired about various other familial, medical, occupational, social and environmental exposures.

Analysis

In both studies, we defined three levels of vitamin exposure during the periconceptional period (−3 to +3 months): (1) nonusers. These include women who reported no use of any vitamin during this period; (2) users during −3 to +3 months. These include women who reported using vitamins both during the period −3 to 0 months and 0 to +3 months; and (3) users during 0 to +3 months only. These include women who reported using vitamins only during the period 0 to +3 months and did not use them before pregnancy.

The association between vitamin use and case-control status was analyzed separately for California and Atlanta using 2 by 2 tables. The odds ratio was used to estimate the relative risk for any protective effect. Odds ratios and exact 95% confidence intervals were derived using a statistical analysis package available at CDC (James, SABER; personal communication). Pooled estimates of the odds ratios and 95% confidence intervals for the two sites combined were obtained using the Mantel-Haenszel procedure [Mantel and Haenszel, 1959]. Further adjustments were made for maternal race/ethnicity (California: white non-Hispanic, Hispanic and other; Atlanta: white and other) and maternal education (<high school, high school and college).

The association was further examined for different types of NTDs (e.g., anencephaly, spina bifida) and also by different types of anomalies accompanying the NTD. The proportion of NTD cases with another defect, such as cleft palate, was compared between vitamin users and nonusers. Odds ratios were also derived as above. Because of small sample sizes and the lack of stability of these odds ratio estimates, only the numbers and proportions are presented here.

RESULTS

Overall Numbers of Controls and Case Subgroups

As shown in Table I, in California, there were 538 NTD cases and 539 controls. In Atlanta, there were 385 NTD cases and 3,029 controls. Most cases were classified as isolated (83% in California and 87% in Atlanta). There were 25 cases with a known syndrome, amniotic bands or holoprosencephaly. The final NTD-multiple group included 65 cases from California and 47 cases from Atlanta.

Association Between Maternal Multivitamin Use and NTD-Multiples

Table I presents the number of mothers who reported no vitamin use, −3 to +3 months use and 0 to +3 months use only for controls and for the different case phenotypic subgroups.

Table II presents odds ratios for the association between maternal vitamin use and of NTD-multiples. Mothers who reported using multivitamins for the period −3 to +3 months were at lower risk of a pregnancy with a NTD-multiple (California: odds ratio = 0.30; Atlanta: odds ratio = 0.48; pooled odds ratio = 0.36, 95% C.I. 0.18–0.72). When the data were further stratified by race/ethnicity, or education, there was no appreciable impact on the magnitude of the odds ratio, indicating that these variables did not act as confounders. For comparison, the crude odds ratios for isolated NTDs were 0.64 (95% C.I. 0.44–0.93) in California and 0.41 (95% C.I. 0.27–0.64) in Atlanta. The numbers of syndromic NTDs were too small to analyze (crude odds ratio 0.79 in California).

Table II also shows that mothers who reported using multivitamins for the period 0 to +3 months only were at lower risk of NTD-multiple (California: odds ratio =

TABLE I. Classification of Periconceptional Vitamin Use in Control and NTD Case Mothers, by Etiology and Associated Defects, California and Atlanta Case-Control Studies

		Maternal vitamin use			
	N	No vitamin use	−3 to +3 months	0 to +3 months only	Other
I. California					
Controls	539	149	94	290	6
All cases	538	207	83	239	9
Syndromes	15	4	2	9	0
Amniotic bands	5	0	2	3	0
Holoprosencephaly	2	1	0	1	0
Multiples	65	32	6	25	2
Isolated	451	170	73	201	7
II. Atlanta					
Controls	3,029	1,179	431	1,172	247
All cases	385	179	27	147	32
Syndromes	1	0	0	1	0
Amniotic bands	1	1	0	0	0
Holoprosencephaly	1	1	0	0	0
Multiples	47	23	4	16	4
Isolated	335	154	23	130	28

TABLE II. Crude and Adjusted Odds Ratios (95% Confidence Interval) for the Association Between Periconceptional Multivitamin Use and the Risk of Neural Tube Defect-Multiples, California and Atlanta Case-Control Studies

	Maternal vitamin use		
	No vitamin use	−3 to +3 months	0 to +3 months only
California	1.0	0.30 (0.11–0.78)	0.40 (0.22–0.73)
Atlanta	1.0	0.48 (0.14–1.46)	0.70 (0.35–1.39)
Adjusted for site[a]	1.0	0.36 (0.18–0.72)	0.51 (0.34–0.78)
Site and education[a]	1.0	0.42 (0.21–0.84)	0.54 (0.35–0.84)
Site and race/ethnicity[a]	1.0	0.45 (0.22–0.92)	0.55 (0.36–0.86)

[a] Stratified by site and adjusted using the Mantel-Haenszel procedure. Maternal education stratified into: <high school; high school and college. Race/ethnicity stratified into white, other (Atlanta); white, Hispanic and other (California).

0.40; Atlanta: odds ratio = 0.70; pooled odds ratio = 0.51, 95% C.I. 0.34–0.78). The magnitude of the risk reduction is less than that of −3 to +3 month use.

Types of Neural Tube Defects and Associated Defects

In Table III, the association between multivitamin use and the risk of NTD-multiples is shown for anencephaly and spina bifida. There was a suggestion of a more marked risk reduction with −3 to +3 months vitamin use for anencephaly-multiples (pooled odds ratio = 0.32) than for spina bifida-multiples (pooled odds ratio = 0.49). The same trend was seen for the 0 to +3 months use only. There were too few cases of encephalocele or other NTDs to draw any conclusions.

In Table IV, the distribution of types of associated defects is shown for case women who were nonvitamin users, −3 to +3 months users, and 0 to +3 months users. Overall, the nonuser group does not have a larger proportion of NTD with specific accompanying anomalies compared to the two other groups. While the numbers are too small to draw firm conclusions, no consistent pattern of associated defects accounts for the overall reduced risk of NTD-multiples in California and Atlanta.

DISCUSSION

This is the first population-based epidemiologic study that attempts to evaluate specifically whether periconceptional multivitamin use may reduce the risk of neural tube defects that occur with other birth defects. Because of the relatively small numbers of NTD-multiples in relation to all NTDs, we pooled data from two population-based case-control studies of neural tube defects. These studies had individually reported an overall reduction in risk of all neural tube defects following periconceptional vitamin use (60% in Atlanta [Mulinare et al., 1988], and 40% in California [Shaw et al., 1995]). However, these studies did not report on the risk reduction of specific patterns of NTDs in combination with other birth defects. The analyses presented in this paper suggest that there is a 60% reduction in the risk of NTD-multiples following periconceptional multivitamin use. This risk reduction was observed in both datasets and persisted after adjustment for potentially confounding factors (race/ethnicity and education). Furthermore, there does not seem to be a specific pattern of associated birth defects that accounts for this overall risk reduction.

TABLE III. Crude and Adjusted Odds Ratios (OR and 95% Limits) for the Association Between Periconceptional Multivitamin Use and the Risk of Neural Tube Defects-Multiples, by Type of Neural Tube Defect,* California and Atlanta Case-Control Studies

	Maternal vitamin use					
	No vitamin use		−3 to +3 months		0 to +3 months only	
	N	OR	N	OR	N	OR
California						
Anencephaly	14	1.0	1	0.11 (0.01–0.84)	9	0.33 (0.13–0.84)
Spina bifida	15	1.0	5	0.53 (0.16–1.62)	14	0.62 (0.28–1.40)
Atlanta						
Anencephaly	10	1.0	2	0.55 (0.08–2.66)	6	0.60 (0.19–1.81)
Spina bifida	11	1.0	0	0.00 (0.00–1.30)	10	0.91 (0.36–2.32)
Combined[a]						
Anencephaly		1.0		0.32 (0.07–1.11)		0.43 (0.22–0.83)
Spina bifida		1.0		0.49 (0.16–1.34)		0.74 (0.42–1.30)

* Numbers of other NTDs too small for statistical analysis.
[a] Stratified by site and adjusted using the Mantel-Haenszel procedure.

TABLE IV. Distribution of Associated Defects Among Infants With Neural Tube Defects-Multiples, by Maternal Vitamin Use, California and Atlanta Case-Control Studies

	Maternal vitamin use					
	No vitamin use		−3 to +3 months		0 to +3 months only	
	N	%	N	%	N	%
California	N = 32		N = 6		N = 25	
Cleft lip ± palate	6	18.8	0	0.0	3	12.0
Cleft palate	2	6.3	2	33.3	3	12.0
All clefts	8	25.0	2	33.3	6	24.0
Omphalocele	2	6.3	3	50.0	3	12.0
Gastrochisis	0	0.0	0	—	0	—
Unspecified abdominal wall defect	2	6.3	0	0.00	2	8.0
Total	4	12.5	3	50.0	5	20.0
Anorectal atresia	3	9.4	3	50.0	7	28.0
Diaphragmatic hernia	5	15.6	0	0.00	1	4.0
Esophageal atresia	1	3.1	0	0.00	0	0.00
Congenital heart defects	6	18.8	1	16.7	3	12.0
Limb deficiency	2	6.3	0	0.00	3	12.0
Renal agenesis	3	9.4	1	16.7	4	16.0
Cystic kidneys	2	6.3	0	0.00	1	4.0
Bladder exstrophy	2	6.3	1	16.7	2	8.0
Other urinary tract defect	4	12.5	1	16.7	5	20.0
Severe genitalia defects	3	9.4	2	33.3	5	20.0
Bowel malrotation	1	3.1	2	33.3	1	4.0
Severe ear defects	1	3.1	0	0.00	0	0.00
Anophthalmia	1	3.1	0	0.00	0	0.00
Atlanta	N = 23		N = 4		N = 16	
Cleft lip ± palate	4	17.4	2	50.0	4	25.0
Cleft palate	1	4.4	0	0.00	0	0.00
All clefts	5	21.7	2	50.0	4	25.0
Omphalocele	10	43.4	0	0.00	4	25.0
Gastrochisis	0	0.0	1	25.0	0	0.0
Unspecified abdominal wall defect	0	0.0	0	0.0	1	6.3
Total	10	43.4	1	25.0	5	31.3
Anorectal atresia	4	17.4	0	0.0	0	0.0
Diaphragmatic hernia	0	0.0	0	0.0	3	18.8
Esophageal atresia	0	0.0	0	0.0	0	0.0
Congenital heart defects	4	17.4	0	0.00	2	12.5
Limb deficiency	5	21.7	1	25.0	3	18.8
Renal agenesis	2	8.7	0	0.0	1	6.3
Cystic kidneys	1	4.4	0	0.00	1	6.3
Bladder exstrophy	2	8.7	0	0.00	0	0.00
Other urinary tract defects	3	13.0	1	25.0	1	6.3
Severe genitalia defects	2	8.7	1	25.0	2	12.5
Bowel malrotation	0	0.0	1	25.0	0	0.0
Severe ear defects	1	4.4	1	25.0	0	0.0
Anophthalmia	0	0.0	0	0.0	0	0.0

In spite of the overall strength of this population-based investigation, it is important to highlight the limitations as well as the methodologic differences between the two individual studies. First, the NTD risk reduction associated with vitamin use may be the result of folic acid itself, other vitamins, or combinations of vitamins with folic acid, or even unmeasured confounding factors. However, the role of folic acid itself in the prevention of NTDs has been inferred from the synthesis of controlled clinical trials and observational studies [CDC, 1992]. It is highly likely that the mechanism involved in the protective effect of multivitamin use in NTD-multiples is similar to the mechanism of the protective effect of all NTDs, and may very well be folic acid itself. Unfortunately, in this study, too few women used vitamin supplements that only contained folic acid or vitamins without folic acid. Thus, the role of other vitamins and minerals in the prevention of neural tube defects and other birth defects should be examined further [Eskes and Steegers-Theunissen, 1994].

Second, differences in the results between the two study sites may be attributable, in part, to diagnostic and ascertainment differences between locations. Atlanta NTD cases spanned over 10 years while California cases were more recent with respect to diagnostic standards and syndrome identification. Also, prenatally diagnosed cases were ascertained only in the California study. These differences may account for the larger proportion of cases with recognized syndromes (mostly chromosomal) compared with Atlanta. The inclusion of undiagnosed syndromes among NTD-multiples cases, if any, can dilute the magnitude of the protective effect (i.e., push odds ratios toward unity) since vitamin use may not reduce the risk of syndromic NTDs. Moreover, the study periods for Atlanta and California are not comparable (unfortunately, more recent vitamin data were not available in Atlanta). There have been well-documented long-term declines in the rates of NTDs in many surveillance systems [Khoury et al., 1982a]. While these methodologic differences undoubtedly can affect the comparability of these two studies, the overall agreement in the protective effect of multivitamins for NTD-multiples found in the two sites is noteworthy.

Third, while there were over 100 NTD-multiples cases from the two sites combined, the numbers of cases with specific patterns of associated birth defects were relatively small. This precludes us from making firm conclusions regarding the specific patterns of NTD-multiples that account for the protective effects of multivitamins.

Finally, the apparent protective effect of first trimester use only found in this study, if not due to unmeasured recall or selection biases, may be due to exposure misclassification. If the timing of the protective effect of folic acid for NTDs is before posterior neural tube closure around day 28, vitamin use after the pregnancy has been detected may be too late to exert such protective effect. Only a proportion of women who reported taking vitamins during the first trimester only took them during the relevant embryonic period; this leads to nondifferential exposure misclassification leading to dilution of the protective effect (i.e., odds ratios closer to unity). In fact, in this study, the odds ratios for first trimester use only are closer to unity than the odds ratios for periconceptional vitamin use. Another possible explanation is that the timing of protective effect of NTD-multiples may go beyond the early gestational period leading to closure of the neural tube. Some investigators have suggested that NTDs with other birth defects may arise because of the rupture of the neural tube after its initial closure [Gardner, 1980; Gardner and Breuer, 1980; Hook, 1992]. Currently, this theory does not have much support. However, it is consistent with the protective effect of vitamin use in the first trimester only against NTD-multiples.

The results of this investigation have two implications in the etiology and prevention of NTDs and birth defects in general. First, the protective effect of folic acid for NTDs appears to extend to NTDs that occur in combination with other anomalies. This adds to the complex and often puzzling epidemiology of neural tube defects. Some investigators have found differences in epidemiologic patterns and familial recurrence risks between NTD-isolated and NTD-multiples. These differences have led to the suggestion that these two groups of NTD may have different causes or pathogeneses. The associations with multivitamins now observed for both groups of NTDs suggest common etiologic links between these NTD-singles and NTD-multiples. Interestingly, in our study, there was no protective effect of vitamins for the syndromic group of NTDs (mainly chromosomal; data shown in Table I), suggesting that the pathogenesis of NTDs associated with chromosomal anomalies is different from other NTDs. This conclusion is tentative because of the very small numbers of NTDs in this group.

The second implication pertains to the potential role of vitamins and folic acid in the prevention of non-NTD birth defects. Vitamins play a fundamental role in human growth and development (e.g., folic acid role in DNA synthesis) and their effects may be apparent in multiple organ system development. Several studies have suggested that NTDs may share similar pathogenetic mechanisms with other types of birth defects such as schisis or midline defects [Czeizel, 1981; Khoury et al., 1988; Opitz and Gilbert, 1982; Opitz, 1993]. NTDs seem to cluster with some other defects in the same infants or families more than by chance [Fraser et al., 1982; Khoury et al., 1982b, 1988]. In addition, recent studies have begun to suggest that there may be a protective effect of periconceptional multivitamin/folic acid for several non-NTD defects including limb deficiencies, oral clefts, conotruncal heart defects and renal anomalies [Shaw et al., 1994a,b; Czeizel, 1993; Stanley and Bower, 1992]. While most of these studies, including the present one, are observational in nature and are affected by potential confounding, selection and recall biases, the randomized controlled clinical trial by Czeizel [1993] showed there is almost a 50% reduction in the occurrence of non-NTD birth defects due to the periconceptional use of multivitamins with folic acid. The numbers of cases with specific types of birth defects were too small to infer which birth defects could be prevented by vitamins.

We suggest that future epidemiologic studies of birth defects assess the role of vitamins (and nutritional factors in general) in the occurrence of a wide variety of birth defects including multiple congenital anomalies. Such studies could have important implications in the primary prevention of birth defects in humans.

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